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Exposure to Fluoride: Adverse Outcome and Toxicity in Review

Universidade Fernando Pessoa

Faculdade de Ciências da Saúde

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degree of Master in Dentistry

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(Jonathan Pierre Raymond Chiniah)

RESUMO

O flúor é amplamente utilizado em Medicina Dentária e a sua eficiência anticariogénica está bem estabelecida. Este trabalho tem como objetivo fazer uma revisão descritiva sobre exposição e toxicidade ao flúor, particularmente para identificar e descrever o possível resultado adverso e efeito tóxico ocorrido por exposição tópica e sistémica. Os dados incluídos na revisão foram sistematicamente pesquisados e coletados em páginas da web (Pubmed, B-on), publicados entre os anos 2000-2017. O flúor pode ser administrado por via sistêmica ou tópica. A fonte mais importante de ingestão de flúor é a água. A maioria dos resultados de estudos da literatura revela alguns efeitos adversos relacionados com exposição a águas fluoradas. A maioria da investigação nesta temática foi efetuada em animais, e revela resultados de toxicidade associados á ingestão de fluor. Contudo, mais estudos são necessários para avaliar resultados adversos associados á exposição ao fluor em seres humanos.

Palavras-Chave: toxicidade fluor, administração flúor, metabolismo flúor, eficácia flúor, "resultados adversos flúor".

ABSTRACT

Fluoride is vastly used in the dental sphere and its efficiency as anticariogenic agent has been well established. This work aims to do a descriptive review on fluoride exposure and toxicity, particularly to identify and describe the possible adverse outcome and toxic effect occurring by topic and systemic exposure to fluoride. Data we used in our review were systematically searched and collected from web pages (PubMed, B-on) published between the years 2000-2017. Fluoride can be administrated via systemic or topical way. The most important source of fluoride ingestion is fluoridated water. Our research suggests that most of the adverse outcomes linked to fluoride is due to fluoridated water. But most of the researches have been made on animals. Even though fluoride ingestion has been linked to toxicity on animals, it needs to be proven on human. More researches need to be done on fluoride effects on human.

Keyword: fluoride toxicity, dental fluoride toxicity, administration of fluoride, metabolism of fluoride, fluoride efficacy, fluoride adverse effects.

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ABREVIATIONS

ppm- parts per million

mM- millimolar

US- United States of America

US EPA- United States Environmental Protection Agency

ATSDR- Agency for Toxic Substances and Disease Registry

WHO- World Health Organisation

FDA- Food and Drug Administration

Mg F/L- milligram Fluoride per litre

Mg/kg- milligram per kilogram

G- gram

Kg- kilogram

IQ- Intelligence quotient

T cell- Lymphocyte T

B cell- Lymphocyte B

IgA- immunoglobulin A

IgM- immunoglobulin M

hECs- human embryonic stem cells

μg kg-1- microgram per kilogram

I- INTRODUCTION

Fluorine is considered one of the most abundant elements in the earth crust. When it is combined with other element, it's produce Fluoride (Barbier et al., 2010), the simplest anion of fluorine. Fluoride is an inorganic, monatomic anion of fluorine with the chemical formula "F−" and is present in the biosphere, atmosphere, hydrosphere and lithosphere (Kanduti et al., 2016). From a chemical point of view, it is the most electronegative and reactive of all the elements due to its small atomic radius. Since it is highly reactive, it is usually bound as inorganic fluoride and not found in its elementary state (Fawell et al., 2006). This ion has been described as an essential nutrient (Dhar and Bhatnagar, 2009). The main sources of fluoride include natural fluoride in food and water, which can be naturally high once has been added (Barbier et al., 2010). In dental environment, fluoride has been vastly used for the treatment of dental caries due to its antimicrobial and anticariogenic properties. Fluoride reduces the demineralization of dental hard tissues by inhibiting the microbial growth, and it promotes the remineralisation of enamel. Due to its efficiency, fluoride has been added to several biomaterials such as composite or bioceramic (Ullah and Zafar, 2015).

Fluoride can be administered either via a topical or a systemic way. Systemic intake of fluoride may come from various sources such as, fluoridated salt, tea or milk, supplement and water fluoridation (Martinez-Mier, 2012). The measure of fluoridating the water was applied in 27 countries which include the USA, Australia and the UK (Peckham and Awofeso, 2014). As for topical intake fluoride can be delivery by toothpaste, mouth rinse, varnish, and gel products (Ullah and Zafar, 2015).

But, as popular and effective fluoride is, there are some adverse outcomes linked to its exposure. Excessive intake of fluoride over a long period can lead to serious public health issues. The main one is fluorosis, which is a global problem, occurring on every continent and affecting millions of people (Song et al., 2014). Dental fluorosis is a hypomineralisation of enamel tissue, due to an excessive intake of fluoride during tooth formation (Martinez-Mier, 2012). Exaggerate ingestion of fluoride may also result in an increased risk of cancer, risk of bone fracture and osteoporosis (Yeung, 2007). Other outcomes that may be related to excessive fluoride intake includes neurological manifestation such as lowering Intelligence quotient (IQ)

level (Barbier et al., 2010), testicular toxicity, kidney damages and more (Song et al., 2014, Wei et al., 2016).

According to this brief presentation, this work aims to do a descriptive review of literature on fluoride exposure and toxicity, particularly to identify and describe the possible adverse outcomes and possible toxic effect occurring by topic and systemic exposure to fluoride, considering also a brief review on fluoride mechanism and efficacy when used in dental hard tissues.

-Materials and methods

Search strategy: searched 2 databases for relevant studies about fluoride exposure; Search was carried in PubMed and B-on, between the years 2000 and 2017. The search of the database yield 700 citations. From the 700 publications, 660 articles did not meet the inclusion criteria. The keywords used were: “fluoride toxicity”, “dental fluoride toxicity”, “administration of fluoride”, “metabolism of fluoride” “fluoride efficacy”, “fluoride adverse effects”. On methodology inclusion criteria were defined: selected articles (manly descriptive and systematic reviews, in vitro and in vivo research publications) which main topic and discussion describe possible fluoride toxicity, adverse outcome due to excessive fluoride intake, metabolism of fluoride, way of administration. Publications that not link to fluoride toxicity, discussing about other type of fluoride topics, were excluded from this review.

II- DEVELOPMENT

1- Expose to fluoride: delivery methods of fluoride brief review

a) Systemic administration

i. Water fluoridation

Drinking water is the largest contributor of the daily fluoride intake (Perumal et al., 2013). It is widely implemented to prevent dental caries. It is a practice that deliberately add fluoride into

the public water supply (Armfield, 2010). It is an ideal public health measure to reduce dental caries, and very effective due to the fact that it does not require daily cooperation from the public (Harding and O'Mullane, 2013). Public water fluoridation was first carried out in the United States of America (US) in 1945, recommended by the World Health Organization (WHO) for the improvement of oral health. Naturally found in fresh water, the concentration of fluoride is compromise between 0.01 ppm to a maximum of 100 ppm, depending on the geographical location and its source. The WHO set a guideline of 1.5 mg F/L (1.5 ppm) as a desirable limit to prevent fluoride toxicity; it allowed also countries to set their own national standard and local guidelines. For example, the US has set its standard to 0.7 ppm, and India 1 ppm (Ullah and Zafar, 2015).

Despite its effectiveness, water fluoridation has not been adopted in many countries as a public health practice (Armfield, 2010). The Netherlands, Germany, and Switzerland have stop it due to concern about safety. No fluoride deficiency disease has ever been documented for humans. Indeed, the basis for setting an "adequate intake" of fluoride rests on the alleged ability of ingested fluoride to prevent tooth decay. However, since it is now known that the effect of fluoride is topical, the notion of an "adequate daily intake" is flawed. One of the key concerns about water fluoridation is the inability to control an individual's dose of ingested fluoride which brings into question the concept of the "optimal dose" (Peckham and Awofeso, 2014).

ii. Salt fluoridation

Salt fluoridation started in 1956 in Zurich. As a daily component of our diet, it is an effective way of administration. Fluoridated salt has been an alternative to water fluoridation in places where it cannot be implemented. Suggested by the WHO, most of the development countries has applied it, including 25 European countries. The main advantage is fluoridated salt is the choice of using it or not, but it is associated with disease such as hypertension (Marthaler, 2013). A recently publication reviewed the progress on policy recommendations in Timor-Leste, since the National Oral Health Survey was done, in 2002 year, the first national survey. Salt Fluoridation was one of the recommendation measures applied, once Timor-Leste faced an urgent set of challenges in oral health and the impact of oral diseases in terms of reducing quality of life and cost of treatment was considerable (Soares et al., 2016)

iii. Fluoride supplement

Devices containing fluoride are used to increase its intake. They slowly release fluoride, helping increased the level of fluoride in the saliva and dental plaque. These devices comprehend muco-adhesive tablets which adhere to the tissue, so the fluoride can act for longer period providing better caries protection. There are also chitosan micro-particles and elastomeric rings (Ullah and Zafar, 2015).

iv. Tea and milk fluoridation

Commonly drink worldwide, tea leaves may contain high levels of fluoride depending of the soil. Indeed, brewed tea may contain up to 6 ppm of fluoride (Waugh et al., 2016). Milk fluoridation is less efficient compared with artificial water fluoridation, due to the fact that the fluoride added to the milk forms insoluble complex that make it difficult to absorb (Fomon et al., 2000).

b) Topical administration

i. Toothpaste

Toothpaste, which have an important role in maintaining oral health, is used daily to remove plaque and debris. Toothpaste containing fluoride were commercialised in the 1970's, and were main source fluoride in places where water fluoridation was not available (Ullah and Zafar, 2015). There are different types of fluoride added to toothpaste: profluoride, that after being delivered, precipitate and release ionic fluoride over time and contribute to the anti-carious efficiency; free ionic fluoride, which interact with the tooth structure, interfere with microbial metabolism and has anti carious efficacy (Carey, 2014). Fluorated toothpaste has a 25% more efficacy in reduction of caries than the non-fluoridated one. However, the concentration of fluoride, the amount of toothpaste used, the duration or frequency of brushing can affect the benefits and efficiency of fluoridated toothpaste. Due to the fact that the concentration of fluoride in toothpaste can vary, it is mandatory that children should be supervised when using toothpaste, when the ingestion can cause toxic effects (Ullah and Zafar, 2015), specially because since it is now known that the effect of fluoride is topical, the notion of an "adequate daily intake" is flawed (Peckham and Awofeso, 2014).

ii. Mouth rinse

Mouth rinse are recommended for patients with a high dental caries susceptibility and can be used in conjunction with toothpaste. Sodium fluoride is the active compound for fluoride delivery in mouth wash. Having the advantage of a lower viscosity than toothpaste, fluoridated mouth rinses can access difficult intra-oral areas such as interproximal posterior surfaces and fissures. Fluoridated mouthwash is recommended for patient with decreased manual dexterity, patient undergoing orthodontic treatment and children over the age of 6 years old with active dental caries risk. For the prevention of fluoride ingestion, fluoridated mouthwash should not be used by under 6 years old people and mentally retarded patients (Ullah and Zafar, 2015).

iii. Fluoride varnish

Fluoride varnish, which has been used since the 1960 for the prevention and control of dental caries must be applied by a qualified oral professional. Varnish deliver fluoride on the surface and subsurface of dental hard tissues, on the carious lesions for a prolonged time. They are indicated to control active lesions, hypersensitive areas of enamel and dentine; and also for the physically or mentally handicapped patients. Its advantages are the fact that its administration is quick and simple, with no special equipment and well tolerated by the patient and considered to be very effective measure (Ullah and Zafar, 2015). However, in the US, the FDA does not approve fluoride varnish use for dental caries prevention due to the insufficient science demonstrating its mechanism of action (Carey, 2014).

iv. Fluoride gel

Fluoride gel are effective in stopping superficial root caries and on reducing dental caries susceptibility, after radiation therapy in patient with cancer, by being incorporated into artificial saliva. They are two types of fluoride gel: stannous fluoride, which is low in concentration 1000 ppm and can be used at home; acidulated phosphate fluoride, which is high in fluoride 10.000 ppm, and need to be apply by a professional (Ullah and Zafar, 2015).

v. Restorative materials

Recently, Cury et al. (2016) review the clinical effectiveness of fluoride dental materials on dental caries control. The authors stated that the mechanism of action of fluoride released from dental materials on carious lesions is similar to that of fluoride found in dentifrices or other vehicles of fluoride delivery. Fluoride-releasing materials are unable to interfere with the

formation of biofilm on dental surfaces adjacent to them or to inhibit acid production by dental biofilms. However, the fluoride released slows down the progression of caries lesions in tooth surfaces adjacent to dental materials. This effect has been clearly shown by in vitro and in situ studies but not in randomized clinical trials. So, as clinical significance, the anti-caries effect of fluoride releasing materials is still not based on clinical evidence, and, in addition, it can be overwhelmed by fluoride delivered from dentifrices (Cury et al., 2016).

2- **Fluoride mechanism and Efficiency**

Fluoride-containing compounds are extremely diverse being not correct to generalise their metabolism, which depends on their structure, reactivity and solubility. The ionic form of fluoride is either generated in the body by biochemical modification of the different fluoride containing compound, or directly ingested; this ionic form is metabolised in a simple manner (Martinez-Mier, 2012). Approximatively 90% of fluoride is absorbed in the intestinal tract, 25% is quickly absorbed in the stomach without the need of any specific enzymes. The majority (77%) of the fluoride absorption occurs in the small intestine and the remaining 10% are excreted in the faeces (Kanduti et al., 2016). The moment fluoride is absorbed, plasma levels increase, attaining its peak concentration 20-60 min after consumption (Kanduti et al., 2016). There are two forms of fluoride in the plasma, one is the ionic fluoride and the other is the non-ionic or bonded fluoride (Martinez-Mier, 2012). The concentration of fluoride in plasma is usually 0.01ppm (Kanduti et al., 2016). When fluoride reaches the plasma, it is quickly deposited in the skeleton and teeth or excreted through the kidney into the urine. The amount of fluoride retained in the skeleton is inversely proportional to the age of the person. Fluoride can be deposited in the adsorbed layers, crystals structures or the bone matrix (Kanduti et al., 2016). Fluoride metabolism can be influenced by different factors. Those main factors are physical activity, hormones, kidney functions, genetic predispositions and the diet (Kanduti et al., 2016).

a) **Fluoride and the Dental caries process**

The dental decay is due to the fermentation of carbohydrate by bacteria covering the teeth. Those Bacteria, which include Streptococcus *mutans*, produce acid when they metabolise carbohydrate. This acid can dissolve the calcium phosphate mineral of the tooth, if this process is not stopped; it will lead to a dental caries lesion. These acids diffuse into the porous enamel

(or dentine if exposed), realising hydrogen ions, freeing calcium and phosphate, leading to hard tissues demineralisation. However the saliva, which has numerous role, neutralise these acids and provides minerals to replace the loss; this replacement is remineralisation(Rošin-Grget and Linčir, 2001).

The mechanisms suggested for the antimicrobial and remineralization roles of fluoride for oral health include, reduction in demineralization by inhibition of microbial growth and metabolism, promotion of remineralization and the formation of the fluorapatite mineral phase (Ca10(PO4)6F2) which is, compared to hydroxyapatite (Ca10(PO4)6(OH)2), more resistant to demineralization and acid dissolution following acid production by bacteria; enzyme inhibition such as reduction of IgA protease synthesis and also, the reduction in extracellular polysaccharide production which helps in decreasing bacterial adherence to dental hard tissues (Ullah and Zafar, 2015).

b) **Inhibition of demineralisation**

When fluoride is present in the solution surrounding the hydroxyapatite crystal, it is absorbed by those crystal and give a protection mechanism against acids. If fluoride is present in the plaque fluid when the bacteria produce acid, it will travel with the acid into the sub-surface of the tooth, absorb into the crystal surface and protect it from being dissolve (Rošin-Grget and Linčir, 2001).

c) **Enhances the remineralisation**

When saliva is saturated with calcium and phosphate provide mineral for the tooth after being attack by acids. Fluoride speeds up this remineralisation process by adsorbing to the surface and attracting calcium ions. This newly formed “veneer” will have a composition between hydroxyapatite and fluorapatite making future acid attack harder to dissolve this remineralized enamel (Masson, 2009). After repeated cycles of demineralisation and remineralisation, the enamel change and becomes more resistant to acids environment (Kanduti et al., 2016).

d) **Inhibition of bacteria plaque**

Fluoride travels true the bacteria cell wall, and acidify the cariogenic bacteria, interfering with its enzymes (Masson, 2009).

3- Fluoride toxicity and possible adverse outcomes

a) Acute toxicity

Acute toxicity occurs due to a single ingestion of a large amount of fluoride. The amount considered lethal is 5 to 10 g of fluoride for a 70-kg adult, but the ingestion of an acute dose is very rare. The symptoms are nausea, bloody vomiting, abdominal pain, diarrhoea and can lead to death in 5 hours if not treated. Today, fluoride poising is mainly due to ingestion of dental products and over fluoridated water (Kanduti et al., 2016).

b) Outcome on hard tissues

If there is excess fluoride ingestion during the tooth formation, there will be an hipomineralisation of the enamel structure and that may lead to dental fluorosis. Clinically, the fluorosed enamel is porous, opaque and with stains. Instead of being normal white translucent, the enamel can have a chalk colour, with cloudy strait or yellowish-brown spot. We can observe fluorosis at 1ppm, and as the doses increase the severity of the signs follows, reaching a pick at 10ppm where the enamel will be fractured after eruption (Perumal et al., 2013). The period when the teeth are the most susceptible is at 2 years old (Peckham and Awofeso, 2014). Excessive fluoride intake (8 ppm), due to fluoridated water for example, can also lead to skeletal fluorosis. This disease leads to structural and functional change in bones, combines with osteoclerosis and osteoporosis (Dhar and Bhatnagar, 2009, Romero et al., 2017)

Table 1- Summary of referential TRVs (toxicity reference values selection) used in the characterization of fluoride effects. These TRVs are related to chronic doses of fluoride (data are extracted from Anses (Anses – French Agency for Food, Environmental and Occupational Health and Safety, 2007) (Guissouma et al., 2017).

REFERENCE	WORK	TRV (TOXICITY REFERENCE VALUES SELECTION)	VALUE (μg kg^{-1} bw per day)	POPULATION	EFFECTS
Health Canada	1996	N/A	122	22 to 26 months	Dental fluorosis
US EPA	1950	RfD	60	children	Dental fluorosis
ATSDR	1990	MRL	60	Postmenopausal woman	Increase in non-vertebral Fractures
Health Canada	1993	N/A	200	adults	Skeletal fluorosis
WHO	2002	N/A	6 mg/day	adults	Skeletal fluorosis

N/A: not available; RfD: reference dose; MRL: Minimum Risk Level; bw: body weight; US EPA-U.S. Environmental Protection Agency;

According to Guissouma and colleagues (2017) citation (Table 1) the TRV values were used to assess the health risk of fluoride to the human body due to oral ingestion and with respect to the duration of exposure (chronic, sub-chronic, or acute). Fluoride levels of 0.5 mgL^{-1} or higher are recommended for prevention of dental cavities (Petersen, 2004, 2003), and a daily intake of 122 mg kg^{-1} body weight of fluoride might cause fluorosis (USEPA, 2005; Gupta, 2011), while more than 200 mg kg^{-1} body weight per day of fluoride might lead to skeletal fluorosis conditions. According to same authors statements there are no harmful effect from daily fluoride intake lower than or equal to the fluoride safety limit of 0.5 mg L^{-1}(Guissouma et al., 2017).

c) Outcome on soft tissue

Chronic ingestion of fluoride has been found to produce deleterious effect in soft tissue such as lungs, kidney or liver in experimental animals. Song et al. (2014) demonstrated that even low dose fluoride induce damage in kidney, by increasing cell apoptosis, damaging the DNA and enhancing histopathological change. Wahluyo et al. (2017) using the USA conform dose of fluoride demonstrated that it induces apoptosis in kidney cell and also affect the proximal tubular epithelial cell. Also excessive fluoride ingestion can cause liver damage by altering enzymatic activities of this organ (Tao et al., 2006). Aydin (2003) conducted a study to analyse the effect of graded doses of Sodium fluoride on the microanatomy of lungs of rats over different periods of time. The authors observed that fluorides induce dose and duration dependent microscopic changes in lung tissue ranging from mild oedema to gross necrosis. With a long-term effect of water fluoridation demonstrated that fluoride damages the lungs, causing loss in the alveolar architecture and inflammation of the lung parenchyma.

d) Outcome on the cerebral region

By penetrating readily into the brain tissue, daily intake of fluoride is likely to produce neurotoxic effect. In a review Choi et al. (2015) exhibit that fluoride exposure to dose below the recommendation can lead to neurotoxic changes, that affect the brain development. In a clinical study on children, Das (2016) concluded that the IQ of the children was affected when they were exposed to elevate fluoride levels in the water. Choi et al. (2012) in a systematic review with the aim investigate the effect of fluoride ingestion and delayed neurobehavioral development found that children who lived in an environment with high fluoride exposure had lower IQ levels than the ones who lived in a low exposure area. Valdez et al. (2017) suggested

that exposure to more than 1.5mg/l during pregnancy could lead to cognitive alterations in children.

e) Outcome on the reproductive system

In an animal study, Sun et al. (2016) discover that fluoride decreased the sperm mobility. Chronic exposure to dose from 50 to 100 ppm (equal to environment levels) caused testicular abnormalities (disorganized cell) and could lead to testicular toxicity (Wei et al., 2016). In a same mind-set, Zhang et al. (2016) using the same dose, shown that fluoride cause excessive apoptosis in testicular cell. Zhang et al. (2017) discover that proteins required for maintaining a normal reproductive function where affected with 100mg/l of fluoride, causing excessive apoptosis and defective autophagy. Fu et al. (2016) demonstrated that even low dose of fluoride (1mM) could disturb the human embryonic stem cells (hECs) and higher dose (2mM) caused supressed proliferation and apoptosis of that cells.

f) Outcome on genes, cells and blood parameters

Fluoride have diverse cell effects; indeed, it alters many mitochondrial cell, cellular respiration, protein transport and cause oxidative stress affecting the cell membrane. It modifies the calcium homeostasis (Sauerheber, 2013), contribute to glucose tolerance and increased blood glucose. Fluoride exposure produce chromosomal aberrations and gene mutations (Barbier et al., 2010). Fluoride alters the cell cycles in the spleen, leading to T, B cell, IgA and IgM reduction (Kuang et al., 2017). Fluoride is a endocrine disruptor and may cause hipotiroidism (Romero et al., 2017). In an animal study, Pereira et al. (2017) revealed that continuous fluoride exposure induce DNA damage due to oxidative stress. Fluoride exerts diverse cellular effects, but fluoride main toxic effect is its action as an enzyme inhibitor. Metabolic, functional and structural damage caused by chronic fluoride have been reported in many tissues. Research data strongly suggest that fluoride inhibits protein secretion and/or synthesis and that it influences distinct signalling pathways involved in proliferation and apoptosis (Table 2 and Table 3) demonstrated its action on diverse cells.

Table 4 and Table 5 shows the main descriptive reviews and clinical research focusing and discussing the possible adverse outcomes of fluoride on the human body. These review publications (Table 4) suggest that fluoride ingestion may cause various effect on endocrine,

dermatological and mostly the neurological system; Fluoride may cause lower IQ level in children.

Table 2- Oxidative stress (increases and decreases regulation) and oxidative damage associated to fluoride exposure on human cells according to in vitro studies (adapted from (Barbier et al., 2010)

MODEL AND DOSE OF FLUORIDE	ENDPOINT	REFERENCE
In vitro (animal cells) Mouse pancreatic beta-cells at 1.35 and 2.5 mM for 12H	↑generation of 02, ↓ activity of superoxide dismutase SOD, ↓ mitochondrial membrane potential (MMP)	E.A. García-Montalvo et al. (2009)
Primary rat hippocampal neurons at 20, 40 and 80 mg/l (1, 2, 4 mM) for 24Hours	↑generation of reactive oxygen species(ROS), ↓ level of activity of SOD, ↑ lipid peroxidation	M. Zhang et al. (2007)
Murine hepatocytes at 100mM for 1Hour	↑generation of ROS, ↑ lipid peroxidation and oxidation of proteins, ↓ level of glutathione (GSH), ↓ level of activity of SOD	Jyotirmoy Ghosh et al. (2008)
In vitro (human cells) Hepatocellular carcinoma cells at 3mM for 6 and 24hours	↓ level of glutathione (GSH),	K.T. Morgan et al. (2002)
Neuroblastoma cells exposed to 0.05-5mM for 24Hours	↑ lipid peroxidation and oxidation of proteins	Q. Gao et al. (2008)
In vivo (humans) Residents from China-endemic area (urine concentration 2mg F/l)	↓ level of activity of SOD, ↑ lipid peroxidation	Q. Chen et al. (2009)
Children with skeletal fluorosis from Indian-endemic area (water concentration of 5.53 mg F/l)	↑Level of ascorbic acid, ↓level of uric acid in plasma ↑Lipid peroxidation, ↓GSH, ↓activities of SOD	S.H. Rao et al. (2001)

mM- millimolar; SOD- superoxide dismutase; MMP- mitochondrial membrane potential; ROS- reactive oxygen species; GSH- glutathione ↑- increase; ↓ decrease;

Table 3 -Regulation of gene expression (increases and decreases regulation) by fluoride exposure on human cells according to in vitro studies (adapted from Barbier et al. (2010).

CELL TYPES AND DOSE OF FLUORIDE	GENES EXPRESSION	REFERENCE
In vitro (animal cells) Mouse pancreatic beta-cells at 1.35, and 2.5 mM for 12 h	↓Insulin	E.A. García-Montalvo et al. (2009)
Primary rat hippocampal neurons at 20, 40 and 80 mg/l (1, 2, 4 mM) for 24H	↓Neural cell adhesion molecules	M. Zhang et al. (2007)
In vitro (human cells) Hepatocellular carcinoma cells at 3mM for 6 and 24h	↓ level of glutathione (GSH),	K.T. Morgan et al. (2002)
Neuroblastoma cells exposed to 0.05-5mM for 24H	↑ lipid peroxidation	Q. Gao et al. (2008)
In vivo (humans) Residents from China-endemic area (urine concentration 2mg F/l)	↓ level of activity of SOD, ↑ lipid peroxidation	Q. Chen et al. (2009)
Children with skeletal fluorosis from Indian-endemic area (water concentration of 5.53 mg F/l)	↑Level of ascorbic acid, ↓level of uric acid in plasma ↑Lipid peroxidation, ↓GSH, ↓activities of SOD	S.H. Rao et al. (2001)

Table 4- Main **review articles** regarding fluoride toxicity/adverse effects, published between the years 2000-2017, in humans.

REFERENCE	YEAR	AIMS	MATHERIAL AND METHODS	RESULTS	CONCLUSIONS
(Romero et al., 2017)	2017	Describe the osteological, neurological, endocrine and dermatological effects of fluoride ingestion.	A scientific revision was made on Medline and ISI.	Fluoride may cause skin hypersensibility; may cause dementia, affect the IQ of children; may diminish the thyroid function; may cause osteoporosis.	Fluoride ingestion have diverse effects on the osteological, neurological, endocrine and dermatological system.
(Peckham and Awofeso, 2014)	2014	Reviews the human health effects of fluoride.	Efficacy and Effectiveness of fluoride the. Adverse Impacts of Fluoride Ingestion on Human Health Its Ethical Arguments.	Given the questionable evidence of benefit and increasing evidence of harm the policy of water fluoridation for the prevention of dental caries should be abandoned in favour of more effective interventions combining communitywide and targeted oral health interventions.	Fluoride has a potential to cause major adverse human health problems, while having only a modest dental caries prevention.
(Choi et al., 2012)	2012	A systematic review and meta-analysis of published studies to investigate the effects of increased fluoride exposure and delayed neurobehavioral development.	Searched the MEDLINE, EMBASE, Water Resources Abstracts, and TOXNET databases through 2011 for eligible studies. They also searched the China National Knowledge Infrastructure (CNKI) database.	The standardized weighted mean difference in IQ score between exposed and reference populations was –0.45 (95% condense interval: –0.56, –0.35) using a random effects model. Thus, children in high fluoride areas had significantly lower IQ scores than those who lived in low fluoride areas.	The results support the possibility of an adverse effect of high fluoride exposure on children's neurodevelopment. Future research should include detailed individual level information on prenatal exposure, neurobehavioral performance, and covariates for adjustment.

Considering the main epidemiologic studies, only one research was found between the year 2000 and 2017. Choi and colleagues (2015) carried out a pilot study to find out if exposure to elevated concentrations of fluoride in water was neurotoxic during development. This epidemiologic study was conducted in 51 first-grade children in southern Sichuan, China, using the fluoride concentration in morning urine after an exposure-free night; fluoride in well-water

source; and measured the dental fluorosis status as indices of past fluoride exposure. Dental fluorosis score was the exposure indicator that had the strongest association with the outcome deficits, and the WISC-IV digit span subtest appeared to be the most sensitive outcome, where moderate and severe fluorosis was associated with a digit span total score difference of −4.28 (95% CI −8.22, −0.33) and backward score with −2.13 (95% CI −4.24, −0.02. Those authors concluded that lifetime fluoride exposures support the notion that fluoride in drinking water may produce developmental neurotoxicity in humans (Choi et al., 2015).

Table 5- Main **clinical research** on fluoride toxicity/adverse effects, published between the years 2000-2017, in humans.

REFERENCE	YEAR	AIM	MATHERIAL AND METHODS	RESULTS	CONCLUSIONS
(Valdez Jiménez et al., 2017)	2017	Evaluate the association between in utero exposure to fluoride (F) and Mental and Psychomotor Development (MDI and PDI)	65 mother-infant pairs. Environmental exposure to fluoride was quantified in tap and bottled water samples. Samples were collected during the 1st, 2nd and 3rd trimester of pregnancy	the MDI showed an inverse association with fluoride levels in maternal urine for the first (b= 19.05, p=0.04) and second trimester (b= 19.34, p = 0.01)	Our data suggests that cognitive alterations in children born from exposed mothers to fluoride could start in early prenatal stages of life.
(El-lethey et al., 2010)	2008	Determine if there are any adverse effects on the developing human brain due to fluoride.	Foetuses from an endemic fluorosis area at the 5th–8th month of gestation were compared with those from a non-endemic area.	The fluoride level in foetus brains from the endemic fluorosis area was 0.28±0.14 μg/g which was higher than the levels in the non-endemic area at 0.19±0.06 μg/g ($p < 0.05$) urkinje cells of foetuses from the endemic fluorosis area were abnormally disorganized and had a thicker granulated layer in the cerebellum. Other dysmorphology, including higher nucleus-cytoplasm ratio of brain cones, hippocampus cones, and Purkinje cone cells, supports the theory that fluoride has an adverse effect on brain development.	The passage of fluorine through the placenta of mothers with chronic fluorosis and its accumulation within the brain of the foetus impacts the developing central nervous system and stunts neuron development.

These in vivo clinical research articles (Table 5) suggest that fluoride may affect the neurological system of the foetus. 2 clinical studies were made. Valdez et al. (2017) with the

aim of associating fluoride exposure to foetus and mental development concluded that there is cognitive alterations in children born from exposed mother to fluoride. El-lethey et al. (2010) concluded that the passage of fluoride to the placenta impact the developing nervous system.

III- DISCUSSION

On the basis of the available evidence done on human, we can make the hypothesis that fluoride has adverse health effects. The main one affects the cerebral region. Fluoride affect the foetus central nervous development (El-lethey et al., 2010) and may cause cognitive alterations in foetuses whose mothers have been exposed to fluoride (Valdez Jiménez et al., 2017). Fluoride may produce developmental neurotoxicity, affecting the IQ of children exposed to it (Choi et al., 2015). But the evidence of fluoride toxicity is mostly done on animals and not on humans. The vast majority of clinical studies are made on rats, demonstrating its toxicity on liver (Song et al., 2014, Wahluyo et al., 2017), its toxicity on the male reproductive system (Sun et al., 2016, Zhang et al., 2017), its toxicity on the immune system (Kuang et al., 2017), and its toxicity on genetics (Campos-Pereira et al., 2017, Barbier et al., 2010). Given the fact that most of the beneficial effect of fluoride are topical, all those adverse outcomes are linked to the ingestion part (Peckham and Awofeso, 2014). Water fluoridation is done in a vast majority of countries, and with WHO letting them set their one guideline, excessive fluoride intake can happen. According to some Peckham and colleagues, available evidence suggests that fluoride has a potential to cause adverse human health problems, while having only a modest dental caries prevention effect. As part of efforts to reduce hazardous fluoride ingestion, the practice of artificial water fluoridation should be reconsidered globally, while safety measures need to be tightened in order to reduce discharge of fluoride compounds into the environment (Peckham and Awofeso, 2014). In order to confirm our hypothesis of fluoride toxicity, more studies need to be done on humans. With all these adverse outcomes, fluoride might be outdated and should be replace with others anticariogenic agents. Newer non-fluoride approaches such as probiotic, Xylitol and biofilms show increasing promise in caries prevention. Fluoride toxicity may be acute or chronic, with effects ranging from cosmetic damage, to disability and even death. With the exception of Dental Fluorosis, Fluoride-related illness is often attributed to other diseases or syndromes (i.e. osteoarthritis for Skeletal Fluorosis, cardiovascular failure for death by acute Fluoride poisoning) making Fluorosis in itself very difficult to track epidemiologically in the absence of an ecosystem health framework (Shah et al., 2013). Most of the research (Annexes

1 to 5) on fluoride adverse effects and toxicity is done in in vitro studies and animals experimental research. More studies are needed to support the evidence of fluoride adverse effects and its toxicology in human being, and also scientific evidence related to analyses of fluoride use or exposure on patient safety and environmental impacts.

IV-CONCLUSION

According to the aims of this descriptive review about exposure to fluoride and toxicology, it is possible to indicate the following conclusion:

- Main exposure to fluoride is done by water fluoridation. Currently at least 30 nations use artificial water fluoridation, which represent hundreds of millions of people. Some countries even increased extensively their water fluoridation, like Australia and Brazil. Excessive fluoride intake can also occur with toothpaste, mouth rinse and tea but on a smaller scale.

- Topical administration is the most effective way of administration fluoride. Fluoride interact directly with the tooth surface, enhancing remineralisation, and inhibiting demineralisation and bacterial plaque. After many cycles of remineralisation and demineralisation, the outer part of the enamel may become more resistant to acidic environment.

-Many adverse outcome and toxic effect can occur when ingesting Fluoride. It can affect the cerebral region, the reproductive system, the hard and soft tissue, the endocrine system, and blood parameters. Most of the dentists know the efficiency of fluoride but they associate toxicity to very high dose of exposure and does not consider that even low dose can have adverse effect on patients. But, even low fluoride dose can be linked to adverse outcomes.

- Fluoride adverse outcomes and toxicity is mainly done by systemic administration and more precisely water fluoridation. In the vast majorities of the articles studied in this review, fluoride was ingested by water fluoridation. An important change would be that the WHO modify its guidelines of 1,5 ppm and allowance of countries to set their own standards. In spite of the limitations of this review due to the fact that most of the articles are done on animals or laboratory studies, and they are not enough on the effect of fluoride on human, it is fundamental that researchers continue and increase their studies of fluoride toxicity on the human body.

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VI. ANNEXE

ANNEX-1: Systematic Review on main articles (published between 2000-2017 years) which main topic is Fluoride adverse effects/toxicology in reviews.

year	Author	Objectives	Math/methods	Results	Conclusion
2017	Romero Verena	Describe the osteological, neurological, endocrine and dermatological effects of fluoride ingestion	A scientific revision was made on Medline and ISI	Fluoride may cause skin hypersensibility; may cause dementia, affect the IQ of children; may diminish the thyroid function; may cause osteoporosis.	Fluoride ingestion have diverse effects on the osteological, neurological, endocrine and dermatological system.
2016	Domen Kanduti, Petra Sterbenk	review the literature about fluoride toxicity to inform medical staff about its safety	Data collected from web pages and documents published from different international institutions	poisoning is mainly due to unsupervised ingestion of products for dental and oral hygiene and over- fluoridated water	Even though fluoride can be toxic in extremely high concentrations, it's topical use is safe
2016	Lucio F Babo Soares	presents a reconfigured set of policies and recommendations for oral health that take into consideration the reasons for low uptake of previous guidance.			
2016	Jaime Aparecido Cury	To describe caries lesions development and the role of fluoride in controlling disease progression; to evaluate whether the use of fluoride-releasing pit and fissure sealants, bonding orthodontic agents and restorative materials	The search was performed on the Cochrane Database of Systematic Reviews and on Medline via Pubmed	The mechanism of action of fluoride released from dental materials on caries is similar to that of fluoride found in dentifrices or other vehicles of fluoride delivery. Fluoride-releasing materials are unable to interfere with the formation of biofilm on dental surfaces adjacent to them or to inhibit acid production by dental biofilms. However, the fluoride released slows down the progression of caries lesions in tooth surfaces adjacent to dental materials	The anti-caries effect of fluoride releasing materials is still not based on clinical evidence, and, in addition, it can be overwhelmed by fluoride delivered from dentifrices.

2015	Rizwan, Ullah	various intraoral delivery methods, the mechanisms of action, toxicity, and the limitations of this treatment.	Water fluoridation Salt fluoridation Miswak and tea Milk fluoridation Toothpaste Mouth rinse Fluoride varnishes Fluoride gel Restorative dental materials	topical, but not systemic, F is beneficial	in order to reduce dental decay in populations with a high caries risk, other measures, such as patient counselling and guidance about oral hygiene and food selection, must be taken in conjunction with the Fluor delivery methods
2014	Stephen Peckham, Niyi Awofeso	reviews the human health effects of fluoride	It looks at Efficacy and Effectiveness of fluoride The Adverse Impacts of Fluoride Ingestion on Human Health The Ethical Arguments	given the questionable evidence of benefit and increasing evidence of harm the policy of water fluoridation for the prevention of dental caries should be abandoned in favour of more effective interventions combining communitywide and targeted oral health interventions.	fluoride has a potential to cause major adverse human health problems, while having only a modest dental caries prevention
2014	Clifton M. Carey	inform the reader about new research and policies related to the use of fluoride for the prevention of dental caries.	Reviews of the current research and recent evidence based systematic reviews on the topics of fluoride are presented. Topics discussed include: updates on community water fluoridation research and policies; available fluoride in dentifrices; fluoride varnish compositions, use, and recommendations; and other fluoride containing dental products		The dental profession is adjusting their recommendations for fluoride use based on current observations of the halo effect and subsequent outcomes. The research community is focused on improving the efficacy of fluoride therapies thus reducing dental caries and lowering the amount of fluoride required for efficacy.

2013	Ekambaram Perumal, Vanaja Paul	Experimentally in animals in order to determine the mechanism involved in the action of F. The reports indicating the chronic harmful effects of F in teeth, bones, heart, liver, kidneys, gastrointestinal tract, lungs, brain, blood, hormones and biochemical parameters of experimental animals and in in vitro studies	It looks at the chronic toxicities of Fluor in teeth, food intake and bones The chronic toxic effect of Fluor on soft tissues Its teratogenic and risk of cancer Looks at the brain, neurotransmitter	The findings presented here indicate that dental fluorosis is the first visible sign of chronic toxic effect of F in experimental animals It is followed by skeletal fluorosis, indicating that a moderate increase in F level than the required level is sufficient to produce harmful effects to teeth and bones. If exposure to toxic concentration of F is continued, it results in an impairment of soft tissues, biochemical parameters, reproduction, and locomotor behavioral activities	A review of the literature that reports the progress of fluorosis in human beings has concluded that no effective therapeutic agent is available for the management of fluorosis. Therefore, future studies in this field should be focused by toxicologists and pharmacologists to investigate antidotes to counteract the harmful effects of F
2012	Anna L. Choi, Guifan Sun	performed a systematic review and meta-analysis of published studies to investigate the effects of increased fluoride exposure and delayed neurobehavioral development	searched the MEDLINE, EMBASE, Water Resources Abstracts, and TOXNET databases through 2011 for eligible studies. We also searched the China National Knowledge Infrastructure (CNKI) database	the standardized weighted mean difference in IQ score between exposed and reference populations was –0.45 (95% condense interval: –0.56, –0.35) using a random effects model. Thus, children in high fluoride areas had significantly lower IQ scores than those who lived in low fluoride areas.	The results support the possibility of an adverse effect of high fluoride exposure on children's neurodevelopment. Future research should include detailed individual level information on prenatal exposure, neurobehavioral performance, and covariates for adjustment.
2011	Joel Berg, DDS; Catherine Gerweck	is consumption of infant formula reconstituted with water that contains various concentrations of fluoride by infants from birth to age 12 months associated with an increased risk of developing enamel fluorosis in the permanent dentition?	MEDLINE search to identify systematic reviews and clinical studies published since the systematic reviews were conducted that addressed the review question.	infants from birth to age 24 months, formula consumption can be associated with an increased risk of developing at least some detectable level of enamel fluorosis	Practitioners should be aware that children are exposed to multiple sources of fluoride during the tooth development period

2010	Olivier Barbier, Laura Arreola-Mendoza	to examine recent findings from our group and others that focus on the molecular mechanisms of the action of inorganic fluoride in several cellular processes with respect to potential physiological and toxicological implications	View the uptake and accumulation of fluoride Its cellular effect The consequence of co-exposure of fluoride and other substances	The studies described above demonstrated that fluoride can interact with a wide range of cellular processes such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, endocytosis, apoptosis/necrosis, and oxidative stress,	In conclusion, this evidence of the positive and negative effects of fluoride needs to be considered along with the ethical, environmental, ecological, financial, and legal issues that surround any decisions about water fluoridation. Any future research into the safety and efficacy of water fluoridation should be carried out with the appropriate methodology to improve the quality of the existing evidence base.
2008	Amid I. Ismail, Hana Hasson	examine evidence regarding the effectiveness of fluoride supplements in preventing caries and their association with dental fluorosis.	the authors searched MEDLINE, the Cochrane Central Register of Controlled Trials, OVID Evidence-based Reviews and EMBASE	One study of primary teeth of children during the first three years of life reported a 47.2 percent reduction in dental caries experience investigators in one trial involving 3- to 6-year-old children found a 43.0 percent difference, and another trial of children in this age group did not find a significant benefit Researchers in several studies involving older children detected a significant reduction in caries increments in permanent teeth with the use of fluoride supplements	There is weak and inconsistent evidence that the use of fluoride supplements prevents dental caries in primary teeth. There is evidence that such supplements prevent caries in permanent teeth. Mild-to-moderate dental fluorosis is a significant side effect.
2007	Australian National Health and Medical Research Council.	The systematic review's research questions relate to the caries-reducing benefits and associated potential health risks of providing fluoride systemically (via addition to water, milk and salt) and the use of topical fluoride agents, such as toothpaste, gel, varnish and mouth rinse	A literature search was undertaken using the Medline and Embase databases the Cochrane Systematic Review and Clinical Trial databases were searched	5418 non-duplicate citations were identified 408 citations were considered potentially eligible for inclusion in the review	Fluoridation of drinking water remains the most effective and socially equitable means of achieving community-wide exposure to the caries prevention effects of fluoride.
2000	John D. B. Featherstone	review the mechanisms of action of fluoride with specific reference to the effect of low levels of fluoride in the fluids in the mouth and to relate this information to the use or misuse of the so-called ''fluoride supplements	It views the carie process, the fluoride mechanism of action, the role of low levels of fluoride in saliva and plaque fluid, the		The anti-caries effects of fluoride are primarily topical for children and for adults. The mechanisms of action of fluoride are (inhibition of demineralization at the crystal sur- faces, enhancement of

			fluoride delivery system		remineralization at the crystal surfaces, and inhibition of bacterial activity. The systemic benefits of fluoride are minimal. Therapeutic levels of fluoride can be achieved from drinking water and topically applied fluoride products. If used, fluoride ‘‘supplements’’ should be employed as a ‘‘topical’’ delivery system by chewing or sucking tablets or lozenges for the maximum possible time before swallowing

ANNEX-2: Systematic Review on main articles (published between 2009-2017 years) which main topic is Fluoride adverse effects/toxicology in Epidemiologic studies.

year	authors	objectives	Mat/methods	results	conclusion
2017	Wiem Guissouma, Othman Hakami	reports a complete survey of the fluoridated tap water in Tunisia and provides the scientific community with a health-risk assessment approach	100 water samples were collected from tap water points located in the 24 Tunisian regions.	The minimum (0.29 mg L) and maximum (1.94 mg L) concentration levels was found in El- Hamma city and in Mareth city respectively. A mixture of these two types of water could be a solution to obtain moderately fluoridated water (~1 mg L). In the other hand, three sites (Gafsa, Tataouin and Mednine) present an exceedance over the Tunisian regulation for fluoride concentrations in drinking water	Our results suggested that approximately 75% of the Tunisian population is at risk for dental decay, 25% have a potential dental fluorosis risk, and 20% might have a skeletal fluorosis risk according to the limits of fluoride in drinking water recommended by WHO
2017	Tewodros Rango	examined the relation between fluoride (F−) concentrations in fingernail clippings and urine and the prevalence and severity of enamel fluorosis (EF) among Ethiopian Rift Valley populations exposed to high levels of F− in drinking water	recorded the EF status of 386 individuals (10 to 50 years old), who consume naturally contaminated groundwater with widely varying F− concentration (0.6–15 mg/L)	5.1 mg/kg (range: 0.5–34 mg/kg) in fingernails and 8.9 mg/L (range: 0.44–34 mg/L) in urine. We show strong positive cor- relations between F− in drinking water and 12-hour urinary excretion (r = 0.74, p b 0.001, n = 287), fingernail F− content (r = 0.6, p b 0.001, n = 258)	Both fingernail and urine measures are good biomarkers for fluoride exposure and EF outcomes

2017	Wiem Guissouma	This study reports a complete survey of the fluoridated tap water taken from 100 water consumption points in Tunisia	The fluoride concentrations in tap water were between 0 and 2.4 mg L 1. Risk assessment of Fluoride exposure was assessed depending on the age of consumers using a four-step method: hazard identification, toxicity reference values selection (TRVs), daily exposure assessment, and risk characterization.	Our findings suggest that approximately 75% of the Tunisian population is at risk for dental decay, 25% have a potential dental fluorosis risk, and 20% might have a skeletal fluorosis risk according to the limits of fluoride in drinking water recommended by WHO.	More investigations are recommended to assess the exposure risk of fluoride in other sources of drinking water such as bottled water
2016	Kousik Da	find out the relationship between fluoride (F) exposure as exposure dose (ED) with dental fluorosis (Waugh et al.), urinary fluoride concentration (UF), intelligence quotient (IQ) and body mass index (BMI)	Fifty groundwater samples were collected from the target area. One hundred forty-nine children belonging to age group 6 to 18 years were considered	exposure rate of Fluor does not show any significant differences (<0.05) among the children of 12 different places. As a result of Fluor exposure, DF cases are mostly found in the order of moderate > severe > mild > very mild > questionable > normal	UF and DF concentration could act as a biomarker of fluoride toxicity.
2016	Declan T. Waugh	Risk Assessment of Fluoride Intake from Tea in the Republic of Ireland	fifty-four brands of the commercially available black tea bag products were purchased and the fluoride level in tea infusions tested by an ion-selective electrode method	According to our risk assessment it is evident that the general population in the RoI is at a high risk of chronic fluoride exposure and associated adverse health effects based on established reference values.	We conclude that the culture of habitual tea drinking in the RoI indicates that the total cumulative dietary fluoride intake in the general population could readily exceed the levels known to cause chronic fluoride intoxication. Evidence suggests that excessive fluoride intake may be contributing to a wide range of adverse health effects

2015	Anna L. Choi, Ying Zhang	Finding if exposure to elevated concentrations of fluoride in water is neurotoxic during development.	We carried out a pilot study of 51 first-grade children in southern Sichuan, China, using the fluoride concentration in morning urine after an exposure-free night; fluoride in well-water source; and dental fluorosis status as indices of past fluoride exposure	Dental fluorosis score was the exposure indicator that had the strongest association with the outcome deficits, and the WISC-IV digit span subtest appeared to be the most sensitive outcome, where moderate and severe fluorosis was associated with a digit span total score difference of −4.28 (95% CI −8.22, −0.33) and backward score with −2.13 (95% CI −4.24, −0.02	lifetime fluoride exposures support the notion that fluoride in drinking water may produce developmental neurotoxicity,
2013	Richard Sauerheber	investigates conditions involved in acute and chronic fluoride toxicity and environmental effects of industrial fluorides added into public water	concentration of the free fluoride ion was mathematically computed at which the solubility of calcium fluoride would be exceeded with calcium concentrations known to be physiologic	Acute and chronic toxicity by fluoride is explain.	Fluoride influences calcium homeostasis. Accidental higher levels of fluoride known to cause acute toxicity.
2013	Máiréad Antoinette Harding[1]	discuss water fluoridation under the following headings: Background, the mode of action, the effectiveness, the risks and benefits, the monitoring of water fluoridation and the legislative nature of providing communities with water fluoridation.			Water fluoridation is an effective safe means of preventing dental caries, reaching all populations, irrespective of the presence of other dental services
2012	A. M. Gbadebo	assess the fluoride levels of groundwater from open wells, consumed by the residents of three communities located in two distinct geological terrains of south- western Nigeria	Fluoride concentration was determined using spectrophotometric technique	groundwater samples from Abeokuta Metropolis (i.e., basement complex terrain) had fluoride content in the range of 0.65 ± 0.21 and 1.20 ± 0.14. These values were found to be lower than the fluoride contents in the	the adults (between the age of 20 and [40 years) showed dental decay than the adolescent (\20 years). This signifies incidence of dental fluorosis by the high fluoride content in the drinking water of the populace

				groundwater samples from Ewekoro peri-urban and Lagos metropolis	
2010	Jason Mathew Armfield, PhD	compared children's decay experience and prevalence between areas with and without water fluoridation in Australia.	Oral health data were obtained from clinical examinations of 128,990 5- to 15-year-old children attending for a regular visit with their respective Australian state or territory School Dental Service in 2002. Water fluoridation status, residence remoteness, and socioeconomic status (SES) were obtained for each children recorded residential postcode area.	Children from every age group had greater caries prevalence and more caries experience in areas with negligible fluoride concentrations in the water (· 0.3 parts per million [ppm]) than in optimally fluoridated areas (· 0.7 ppm).	This study demonstrates the continued community effectiveness of water fluoridation and provides support for the extension of this important oral health intervention to populations currently without access to fluoridated water.
2009	John J. Warren, Steven M Levy	present longitudinal fluoride intake data for children free of dental fluorosis in the early-erupting permanent dentition and free of dental caries in both the primary and early-erupting permanent teeth as an estimate of optimal fluoride intake	data on fluoride ingestion were obtained from parents of 602 Iowa Fluoride Study children through periodic questionnaires at the ages of 6 weeks; 3, 6, 9, 12, 16, 20, 24, 28, 32, and 36 months; and then at 6-month intervals thereafter.	the estimated mean daily fluoride intake for those children with no caries history and no fluorosis at age 9 years was at, or below, 0.05 mg F/kg bw for nearly all-time points through the first 48 months of life, and this level declined thereafter. Children with caries had generally slightly less intakes, whereas those with fluorosis generally had slightly higher intakes	Given the overlap among caries/fluorosis groups in mean fluoride intake and extreme variability in individual fluoride intakes, firmly recommending an "optimal" fluoride intake is problematic

ANNEX-3: Systematic Review on main articles (published between 2014-2017 years) which main topic is Fluoride adverse effects/toxicology in *In Vitro* studies.

year	authors	objectives	Mat/methods	results	conclusion
2017	Moumita Dutta, Prem Rajak	explore the toxic impact of chronic NaF exposure on a non-target organism, Drosophila melanogaster	larvae exposed to different concentrations of NaF through food	The larvae showed a significant increase in HSP70 expression both qualitatively and quantitatively. The altered tail length and tail intensity in Comet assay validate the increased DNA damage in treated larvae. The activity of AChE, oxidative stress marker enzymes, phase I and phase II detoxifying enzymes were found to be significantly inhibited in the treated larvae when compared to control though there was no evidence of dose dependent change in each case. The alterations in the mentioned parameters can be due to increased body Fluoride ion (F) concentration	the results suggest that D. melanogaster manifest prominent toxic response when subjected to chronic exposure to sub-lethal NaF concentrations.
2017	S. WAHLUYO, K. ISMIYATIN	this study was aimed to investigate the NaF exposure effects on the growth of ameloblasts and kidney proximal tubular cells	adult male healthy rats were used as experiment models, divided into control and NaF-induced groups. The expression of amelogenin, Bcl-2, and caspase-3 were significantly different in the control and NaF-induced group ($P < 0.05$).	There was no correlation among these proteins in the control group but significant correlation in the NaF-induced group ($r = 0.694$). There was a significant correlation in proximal tubular cells, as seen from the increase of caspase-3 in the NaF-induced group ($r = 0.715$).	Fluoride exposure causes cell damage and cell death, and thereby affects the growth of ameloblast cells in the rat teeth and the existence of proximal tubular epithelial cells in the rat kidneys

2016	Xin Fu, Fang-Nan Xie	investigated the effects of sodium fluoride (NaF) on the proliferation, differentiation and viability of H9 hESCs	Chemicals, Cell culture, EB formation, PCR, Cell proliferation and viability	High-dose NaF caused the death of hESCs via apoptosis in a caspase-mediated	NaF might interfere with early human embryogenesis by disturbing the specification of the three germ layers as well as osteogenic lineage commitment and that high-dose NaF could cause apoptosis through a JNK-dependent pathway in hESCs.
2016	Zilong Sun, Wen Zhan	View the effect of fluoride exposure on ATP generation in sperm mobility	20 healthy male mice were orally administrated with 0, 25, 50, and 100 mg L^{-1} NaF for 90 d	fluoride ingestion significantly reduced sperm count, survival, as well as mobility	decreased sperm motility induced by fluoride may result from low ATP generation due to the disturbed ETC in sperm mitochondrial
2016	ShunZhang, Qiang Niu	investigate the roles of apoptosis and autophagy in testicular toxicity of fluoride	rats were exposed to 25, 50, or 100 mg/L sodium fluoride (NaF) via drinking water from pre-pregnancy to post-puberty	NaF exposure induced an enhanced testicular apoptosis	excessive apoptosis and defective autophagy in the aggravation of testicular damage due to fluoride exposure
2014	Cardoso	clarify the mechanisms of action of fluoridated acidic liquid dentifrices against dental caries.	enamel specimens were submitted to a pH-cycling model, treated with distinct dentifrices (0, 550 mgF/g pH 4.5	In vivo, the reduction of the pH was able to significantly increase plaque F uptake, leading to similar levels as those found for the neutral dentifrice containing twice [F] -the reduction of the pH had a partial preventive effect on subsurface hardness loss	The results obtained from in vitro studies whose design does not include the presence of dental plaque should be interpreted with caution

			and pH 7.0, 1100 or 5000 mgF/g pH 7.0). -2-to-4-year-old children who had been using liquid dentifrices for 6 months (550 mgF/g pH 4.5 or pH 7.0 or 1100 mgF/g pH 7.0) had their plaque samples collected 5 and 60 min after the last brushing	only. [F] had a significant influence on the deposition of fluoride, surface and subsurface hardness lo	
2014	Guo Hua Song, Ji Ping Gao	determine the effects of NaF treatment on renal cell apoptosis, DNA damage, and the protein expression levels of cytosolic cytochrome C (Cyt C) and cleaved caspases 9, 8, and 3	Male Sprague-Dawley rats were divided randomly into four groups (control, low fluoride, medium fluoride, and high fluoride) and administered 0, 50, 100, and 200 mg/L of NaF, respectively, via drinking water for 120 days	NaF treatment increased apoptosis and DNA damage. In addition, NaF treatment increased the protein expression levels of cytosolic Cyt C and cleaved caspases 9, 8, and 3	NaF induces apoptosis in the kidney of rats through caspase-mediated pathway, and DNA damage may be involved in this process.

ANNEX-4: Systematic Review on main articles (published between 2003-2017 years) which main topic is Fluoride adverse effects/toxicology in **Laboratorial animal studies.**

year	author	Objectives	Math/methods	results	conclusion
2017	Ping Kuang, Huidan Deng	This study was designed to evaluate the toxic effects of NaF on the splenic development of mice in vivo by observing histopathological lesions, changes of splenic growth index (GI), T and B cells, immunoglobulin A (Borysewicz-Lewicka et al.), immunoglobulin G (IgG) and immunoglobulin M (IgM) contents	240 ICR mice were equally allocated into four groups with intragastric administration of distilled water in the control group and 12, 24, 48 mg/kg NaF solution in the experimental groups for 42 days	The results showed that NaF in 12 mg/kg and over caused the toxic effects on splenic development, which was characterized by reducing growth index and lymphocytes in the white and red pulp histopathologically, increasing cell percentages of the G0/G1 phase and decreasing cell percentages of the S phase, and reducing T cells and B cells as well as IgA, IgG, and IgM contents when compared with those in the control group	Toxic effects impaired the splenic cellular immunity and humoral immunity due to the reduction of T and B cell population and activity. Cell cycle arrest is the molecular basis of NaF-caused toxic effects on the splenic development.
2017	Jianhai Zhang1 & Yuchen Zhu	determine expression levels of P450, cAMP, CREM proteins in testes of rats to understand the mechanism of fluoride toxicity on spermatogenese	administered 100 mg NaF/L for 2 weeks via drinking water to rats	P450 expression was decreased while CREM and ACT expression was increased in the fluoride group, compared to the control	fluoride can impair male reproduction by affecting expression of P450, CREM, and ACT in the testes.
2017	Ping Kuang	evaluate the toxic effects of NaF on the splenic development of mice in vivo	240 ICR mice were equally allocated into four groups with intragastric administration of distilled water in the control group and 12, 24, 48 mg/kg NaF solution in the experimental groups for 42 days.	NaF in 12 mg/kg and over caused the toxic effects on splenic development, which was characterized by reducing growth index and lymphocytes in the white and red pulp histopathologically, increasing cell percentages of the G0/G1 phase and decreasing cell percentages of the S phase, and reducing T cells and B cells as well as IgA, IgG, and IgM contents when compared with those in the control group	Toxic effects impaired the splenic cellular immunity and humoral immunity due to the reduction of T and B cell population and activity. Cell cycle arrest is the molecular basis of NaF-caused toxic effects on the splenic development.

2017	F.D. Campos-Pereira	reported a probable link to oxidative stress, DNA damage and apoptosis induced by fluoride in rat hepatocytes.	in vivo study administering three doses of fluoride by gavage given to rats for 60 days.	Our results revealed the genotoxic potential of fluoride. Oxidative stress induction was confirmed and is probably associated to DNA damage. Cell death events such as empty nuclear spaces, cytoplasm degeneration	prolonged fluoride intake at chosen concentrations caused imbalance of the cellular oxidative state, affected DNA and disrupted cellular homeostasis. It is recommended that fluoride supplementation requires a fresh consideration in light of the current study.
2016	Ruifen Wei, Guangying Luo	evaluate the sperm quality after Fluoride exposure	Healthy male mice were randomly divided into four groups with sodium fluoride (NaF) at 0, 25, 50, 100 mg/L in the drinking water for 180 days	increased percentage of spermatozoa abnormality was found in mice exposed to 50 and 100 mg/L NaF.	testicular inflammatory response could contribute to chronic F exposure induced testicular toxicity in mice
2016	Şirin Güner	evaluated dental fluorosis of the incisors and immunoreactivity in the brain tissues of rats given chronic fluoride doses pre- and postnatally	Female rats were given drinking water with 0, 30 or 100 ppm fluoride ad libitum throughout gestation and the nursing period. The upper and lower incisors were collected Cortical, hippocampal and cerebellar brain samples were evaluated	All fluoride-treated pups were born with low body weight ($p = 0.001$). All animals from the fluoride groups had enamel fluorosis with defects of various degrees	rats with dental fluorosis had catalase immunoreactivity in the brain tissues, which may reflect the neurobehavioral toxicity of fluoride
2015	Jing Ma, Fei Liu	examine whether exposing immature mice to fluoride would modify the peripheral pain	fluoride was given to mice in different concentration (0	Hyperalgesia in fluoride exposure mice was exhibited in the Von Frey hair test, hot plate test and formalin test	early developmental fluoride exposure may lower the basal pain threshold and be

		sensitivity or even cause a pain syndrome. We gave developmental fluoride exposure to mice in different concentration (0 mg/L, 50 mg/L and 100 mg/L) and evaluated their basal pain threshold.	mg/L, 50 mg/L and 100 mg/L) and evaluated their basal pain threshold Von Frey hair test, hot plate test and formalin test were conducted the expression of hippocampal brain-derived neurotrophic factor (BDNF) was also evaluated	the expression of BDNF was significantly higher than that of control group	associated with the increasing of BDNF expression in hippocampus.
2014	Guo Hua Song, Ji Ping Gao	determine the effects of NaF treatment on renal cell apoptosis, DNA damage, and the protein expression levels of cytosolic cytochrome C (Cyt C) and cleaved caspases 9, 8, and 3	Male Sprague-Dawley rats were divided randomly into four groups (control, low fluoride, medium fluoride, and high fluoride) and administered 0, 50, 100, and 200 mg/L of NaF, respectively, via drinking water for 120 days	NaF treatment increased apoptosis and DNA damage. In addition, NaF treatment increased the protein expression levels of cytosolic Cyt C and cleaved caspases 9, 8, and 3	NaF induces apoptosis in the kidney of rats through caspase-mediated pathway, and DNA damage may be involved in this process.
2011	Piler Mahaboob Basha, Narayanaswamy Madhusudhan	Fluoride effect in developmental stages of life	rats were exposed to 50 and 150 ppm fluoride in drinking water during gestation and post gestation After birth, the pups born to the experimental	The results significantly ($P < 0.05$) demonstrated the effect of fluoride through exacerbated oxidative damage and disrupted antioxidant homeostasis, leading to altered neuronal integrity.	The administration of antioxidants vitamin E, vitamin C, selenium and zinc produced a promising accost and timely intervention to the aggravated impairment during highly vulnerable early stage of life.

			animals were administered daily with selected antioxidants for 21 consecutive days		
2008	Li Du, Changwu Wan	determine if there are any adverse effects on the developing human brain	foetuses from an endemic fluorosis area at the 5th–8th month of gestation were compared with those from a non-endemic area.	The fluoride level in foetus brains from the endemic fluorosis area was 0.28±0.14 μg/g which was higher than the levels in the non-endemic area at 0.19±0.06 μg/g ($p < 0.05$) urkinje cells of foetuses from the endemic fluorosis area were abnormally disorganized and had a thicker granulated layer in the cerebellum. Other dysmorphology, including higher nucleus-cytoplasm ratio of brain cones, hippocampus cones, and Purkinje cone cells, supports the theory that fluoride has an adverse effect on brain development.	the passage of fluorine through the placenta of mothers with chronic fluorosis and its accumulation within the brain of the foetus impacts the developing central nervous system and stunts neuron development.
2007	Aline de Lima Leite, Joel Ferreira Santiago Júnior	the purpose of this study was to investigate if exposure to single high doses of sodium fluoride could cause DNA damages in liver, kidney, urinary bladder and thyroid gland of rats by means of the single cell gel	the potential DNA damage associated with exposure to fluoride was assessed in cells of blood, liver, kidney, thyroid gland and urinary bladder by the single cell gel (comet) assay. Male Wistar rats aging 75 days were distributed into seven groups: Groups 1 (control), 2, 3, 4, 5, 6 and 7 received 0 (deionized water), 10, 20, 40, 60, 80 and 100 mgF/Kg	The level of DNA strand breaks did not increase in all organs evaluated and at all doses of NaF tested, as depicted by the mean tail moment	our results suggest that oral exposure to NaF did not result in systemic genotoxic effect in multiple organs related to fluoride toxicity.

			body weight from sodium fluoride (NaF), respectively, by gastrogavage. These groups were killed at 2 h after the administration of the fluoride doses		
2004	Xin TAO, Zi Rong XU	assessed the effects of excessive fluoride ingestion on growth performance, serum indexes and antioxidant systems in growing pigs.	ninety-six growing pigs were used to evaluate the effects of fluoride. four dietary treatments were formulated by supplementing fluorine (as NaF) to provide the following added fluorine levels: 0, 50, 100 and 150 mg/kg	pigs consuming diets with 100 and 150 mg/kg fluorine added had poor growth performance, and most serum biochemical indexes were significantly altered compared to the control ($P < 0.05$). On the other hand, serum and liver MDA concentration significantly increased due to the addition of 150 mg/kg fluorine ($P < 0.05$). T-AOC levels and the activities of SOD, GSH-PX, CAT and GST of serum and liver in fluoride added groups decreased, most of which altered significantly ($P < 0.05$)	These results indicated that excessive fluoride ingestion had an adverse effect on animal health and performance.
2003	Gülsen Aydin, Ekrem Çiçek,	investigate the possible effects of multigenerational administration of sodium fluoride (NaF) via drinking water on lung tissue morphology and biochemistry and body and lung weight	Twenty-eight pregnant rats were selected for the experiment, divided into four groups of seven rats given 1 (control group), 10, 50 and 100 mg l−1 NaF in drinking water during the gestation period. After gestation, the rats had 165 pups in total	Histological findings showed alveolar congestion, alveolar cell hyperplasia and necrosis	the lung tissues were damaged, there was emphysema and inflammation of lung parenchyma associated with loss of alveolar architecture and the degree of lung damage seemed to correlate with the increased dosage of fluoride ingested via fluorated water.

ANNEX-5: Systematic Review on main articles (published between 2007-2017 years) which main topic is Fluoride adverse effects/toxicology in CLINICAL TRIALS

year	author	Objectives	Math/methods	results	conclusion
2017	L. Valdez Jiménez	Evaluate the association between in utero exposure to fluoride (F) and Mental and Psychomotor Development (MDI and PDI	65 mother-infant pairs. Environmental exposure to fluoride was quantified in tap and bottled water samples. Samples were collected during the 1st, 2nd and 3rd trimester of pregnancy	the MDI showed an inverse association with fluoride levels in maternal urine for the first (b= 19.05, p=0.04) and second trimester (b= 19.34, p = 0.01)	Our data suggests that cognitive alterations in children born from exposed mothers to fluoride could start in early prenatal stages of life.
2012	Elsa Vasquez, Graciela Zegarra	characterize the short-term pharmacokinetics of fluoride and silver in serum, subsequent to oral ingestion of DSF from topical application to teeth of adults.	this preliminary study determined the applied doses (3 teeth treated), maximum serum concentrations, and time to maximum serum concentration for fluoride and silver in 6 adults over 4 h	Over the 4 hours observation period, the mean maximum serum concentrations were 1.86 μmol/L for fluoride and 206 nmol/L for silver. These maximums were reached 3.0 h and 2.5 h for fluoride and silver, respectively.	Fluoride exposure was below the U.S. Environmental Protection Agency (EPA) oral reference dose. Silver exposure exceeded the EPA oral reference dose for cumulative daily exposure over a lifetime, but for occasional use was well below concentrations associated with toxicity. This preliminary study suggests that serum concentrations of fluoride and silver after topical application of DSF should pose little toxicity risk when used in adults.

2008	Li Du, Changwu Wan	determine if there are any adverse effects on the developing human brain	foetuses from an endemic fluorosis area at the 5th–8th month of gestation were compared with those from a non-endemic area.	The fluoride level in foetus brains from the endemic fluorosis area was 0.28±0.14 μg/g which was higher than the levels in the non-endemic area at 0.19±0.06 μg/g ($p < 0.05$) urkinje cells of foetuses from the endemic fluorosis area were abnormally disorganized and had a thicker granulated layer in the cerebellum. Other dysmorphology, including higher nucleus-cytoplasm ratio of brain cones, hippocampus cones, and Purkinje cone cells, supports the theory that fluoride has an adverse effect on brain development.	the passage of fluorine through the placenta of mothers with chronic fluorosis and its accumulation within the brain of the foetus impacts the developing central nervous system and stunts neuron development.
2007	Maria Borysewicz-Lewicka, Justyna Opydo-Szymaczek	evaluate the amount of fluoride remaining in the oral cavity of children after brushing with fluoride gel (1.25% F)	The study involved six groups of 7-year-old and six groups of 11-year-old children	No statistically significant difference was found between the amount of fluorides that remained in the oral cavity of younger and older age group (1.2 and 1.3 mg, respectively; $p>0.05$) the amount of fluorides swallowed during the procedure in both age groups proves to be within acceptable limit, as far as risk of acute poisoning symptoms is concerned. The individual daily fluoride exposure during the day of procedure seems to be twice as high compared to average fluoride intake from diet and dentifrice, and it does not exceed Tolerable Upper Intake Level for children more than 8	In younger children, it seems justifiable to reduce the amount of the preparation applied on a toothbrush, especially when daily use of the gel is recommended